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14. ABSTRACT

TMPRSS2-ERG translocation is involve d in about 60% of prostate canc ers. The translocation leads to overexpression of ERG which prom otes cell proliferati on and abrogates prostate epithelial differentiation. Suppression of ERG expression represen ts a major way to treat prostate can cers. However, there are no effective means to inhibit ERG expression. Although siRNA can deplet e ERG expression, the big challenge is to deliver siRNA into prostate cancer cells. The mRNA stability, translation efficiency and protein stability of ERG are under strict control to maintain the ERG protein level. Here we report the establishment of a cell line which can be used to identify small molecules which inhibit ERG expression by targeting its post-transcriptional process. Using this cell line, we perfor med high-throughput screening and identif ied Gambogic acid which suppr esses ERG expression in ERG-overexpressing prostate cancer cells.

15. SUBJECT TERMS
Prostate carcinoma; ERG; small molecules, post-transcriptional.

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INTRODUCTION

TMPRSS2-ERG translocation is involved in about 60% of prostate cancers. The translocation leads to overexpression of ERG which promotes cell proliferation and abrogates prostate epithelial differentiation. Suppression of ERG expression represents a major way to treat prostate cancers. However, we do not have effective means to inhibit ERG expression. Although siRNA can deplete ERG expression, the big challenge is to deliver siRNA into prostate cancer cells. The mRNA stability, translation efficiency and protein stability of ERG are under strict control to maintain the ERG protein level. The objectives of this proposed research are to identify small molecules which inhibit the expression of ERG by targeting its post-transcriptional process.

BODY

Task 1. Establish a prostate cancer reporter cell line for screening.

We at first generated a vector which expresses a transcript composed of coding region for luciferase and ERG fusion protein and the 3'UTR of ERG (Fig. 1) with pCDNA3.1. The inclusion of the reporter luciferase cDNA into ERG cDNA allowed the identification of small molecules which affect ERG mRNA stability and translation efficiency. By using the in-frame fusion of luciferase with ERG cDNA, chemicals affecting ERG protein stability can also be identified. To establish a cell line expressing luciferase-ERG fusion protein, the vector along pRL-CMV-Rluc expressing Renilla luciferase gene was transfected into LNCaP cells. The pRL-CMV-Rluc served as an internal control. The cells were treated with G418. Individual clones were isolated, expanded, and examined for the expression of two different luciferases. A clone expressing both Firefly luciferase and Renilla luciferase was selected for further studies.

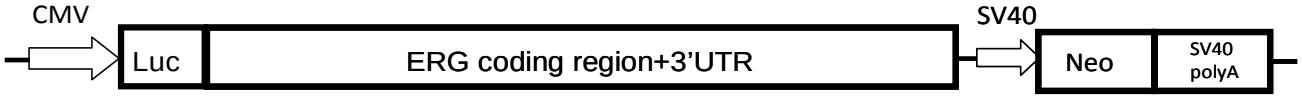
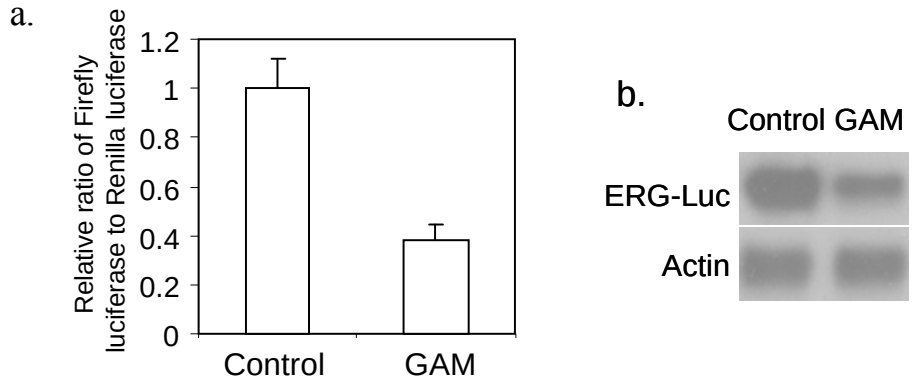


Fig. 1. The structure of vector expressing Luciferase and ERG fusion protein.

Task 2. Perform high throughput screening for small molecules.

The screening was performed in our university core facility. Ten thousand reporter cells were seeded per well in 384 well-plates and were treated with the individual chemical at 10 μM for 24 h. The dual luciferase activities were measured. The ratio of Firefly to Renilla luciferase activity was calculated. The ratio value decreased to 40% of the value from the control cells were considered as a hit and selected for further analysis. From a total of 5 hits, we identified Gambogic acid which consistently suppressed Firefly luciferase activity compared with Renilla luciferase activity (Fig. 2a). We confirmed that Gambogic acid decreased the protein level of ERG-Luciferase fusion protein (Fig. 2b).

Fig. 2. (a). Gambogic acid suppresses the activity of Firefly luciferase. Cells were treated with control vehicle or Gambogic acid (GAM) for 24h. The Firefly and Renilla luciferase activities were measured and the ratios were calculated. (b). Gambogic acid (GAM) decreased the protein level of ERG-Luciferase (ERG-Luc) fusion protein, as shown by Western blot.



Task 3. Test if the identified molecules can inhibit the proliferation of prostate cancers by inhibiting ERG protein expression.

VCaP cells which carry TMPRSS2-ERG translocation were treated with Gambogic acid or control vehicle for 48h. Real time RT-PCR for ERG revealed that ERG mRNA was not changed (Fig. 3a), indicating that the compound does not affect the ERG mRNA stability. However, Western blot revealed that ERG protein was reduced by the treatment (Fig. 3b). Pulse-chase experiment revealed that ERG protein is less stable when treated with Gambogic acid (Fig. 3c), indicating that the compound acts through down-regulating the protein stability.

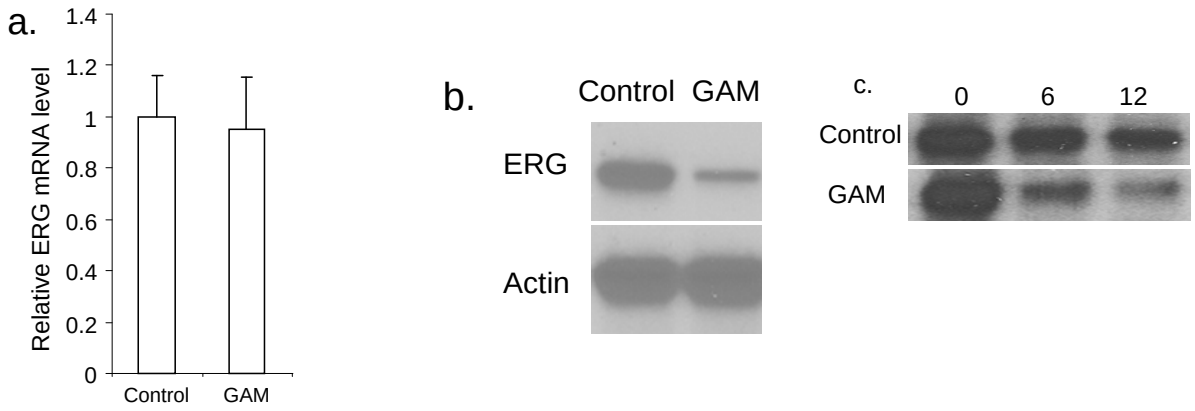


Fig. 3. a). ERG mRNA levels are not affected by the treatment of Gambogic acid (GAM), as revealed by real-time RT-PCR. b). ERG protein is reduced by the treatment of Gambogic acid, as shown by Western blot. c). ERG protein is less stable with the treatment of Gambogic acid (GAM), as revealed by pulse-chase analysis. S^{35} labeled ERG was precipitated by anti-ERG at several time points (0, 6h, 12h) following the labeling with S^{35} for 30 minutes.

KEY RESEARCH ACCOMPLISHMENTS

* We report the establishm ent of a cell line which can be used to identify small mole cules inhibiting ERG expression.

* We performed high-throughput screening and identified Gambogic acid which suppresses ERG-Luc expression.

* We found that Gambogic acid inhibits the expression of ERG in ERG-overexpressing prostate cancer cells.
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REPORTABLE OUTCOMES

None

CONCLUSIONS

We report the establishment of a cell line which can be used to identify small molecules which inhibit ERG expression. Using this cell line, we performed high-throughput screening and identified Gambogic acid which suppresses ERG-Luc fusion protein expression. We found that Gambogic acid can suppress ERG expression in ERG overexpressing prostate cancer cells.

REFERENCES

None

APPENDICES

None.